UNIVERSIDADE FEDERAL DO PARANÁ

LAIZA CABRAL DE FARIA

COMUNIDADE BACTERIANA ASSOCIADA AO CORAL-SOL, *TUBASTRAEA COCCINEA* (SCLERACTINIA: DENDROPHYLLIIDAE), DA COSTA DO BRASIL

Pontal do Paraná

2020

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COMUNIDADE BACTERIANA ASSOCIADA AO CORAL-SOL, *TUBASTRAEA COCCINEA* (SCLERACTINIA: DENDROPHYLLIIDAE), DA COSTA DO BRASIL

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Sistemas Costeiros e Oceânicos, no Curso de Pós-Graduação em Sistemas e Costeiros e Oceânicos, Setor de Ciências da Terra, da Universidade Federal do Paraná.
Orientador: Prof. Dr. Marcelo Visentini Kitahara

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em SISTEMAS COSTEIROS E OCEÂNICOS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **LAIZA CABRAL DE FARIA**, intitulada: **COMUNIDADE BACTERIANA ASSOCIADA AO CORAL-SOL, TUBASTRAEA COCCINEAS(SCLERACTINIA: DENDROPHYLLIDAE). DA COSTA DO BRASIL**, sob orientação do Prof. Dr. MARCELO VISENTINI KITAHARA, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
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Curitiba, 26 de Março de 2020.

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RESUMO

Os microrganismos têm um papel fundamental na sobrevivência dos corais, sendo responsáveis por parte da nutrição dos corais e controle de patógenos. *Tubastraea coccinea*, conhecido popularmente como coral-sol, é um coral pétreo azooxantelado considerado invasivo no litoral brasileiro. Devido à falta de predadores naturais, reprodução rápida e extensos mecanismos de defesa, o coral-sol se espalhou rapidamente ao longo da costa brasileira, ameaçando espécies nativas e modificando as funções do ecossistema. Bactérias e Arqueia associadas a *T. coccinea* de 15 pontos de amostra, ao longo de um gradiente de latitudinal de 3500 km foram identificadas usando a plataforma de sequenciamento *MiSeq* de sequencias parciais do gene 16S rDNA. Um total de 2660 OTUs (Unidade Taxonômica Operacional) em nível de gênero foram identificadas em todas as 45 amostras. As bactérias representaram 99,5% e as arqueias, apenas 0,5% de todas as sequências. O filo *Proteobacteria* foi a mais abundante, totalizando mais de 75% de todas as amostras. Para entender se as condições ambientais podem moldar a composição microbiana, foram testadas longitude, latitude, NO_3, PO_4, Fe, temperatura e clorofila. Entretanto, nenhum dos fatores ambientais testados pôde explicar a composição procariótica. Nossos resultados indicam que a comunidade bacteriana de *T. coccinea* pode ser mais influenciada pela poluição por hidrocarbonetos e o substrato no qual as colônias estavam fixadas do que pelos fatores ambientais testados.

Palavras-chave: Atlântico Sul; Gradiente Latitudinal; Simbiose; Metabarcoding; 16S rDNA

ABSTRACT

Microorganisms have a key role in coral growth and survival, being responsible for part of coral nutrition and pathogen control. The orange sun coral *Tubastraea coccinea*, is an azooxanthellate stony coral invasive in the Central Atlantic (Canary Islands to Brazil). Due to the lack of natural predators, rapid reproduction and extensive defense mechanisms, the sun coral has spread rapidly along the Brazilian coastline, threatening native species and modifying ecosystem functions. *Bacteria* and *Archaea* associated with *T. coccinea* from 15 sites along a 3500km latitudinal gradient were identified using *MiSeq* sequencing of partial 16S rDNA genes. A total of 2 660 OTUs (Operational Taxonomic Unit) at genus level were identified in all samples. *Bacteria* accounted for 99.5% and *Archaea* for only 0.5% of all sequences. The phylum *Proteobacteria* was the most abundant, with more than 75% of all samples. To understand if environmental conditions can shape the microbial composition of *T. coccinea*, longitude, latitude, NO3, PO4, Fe, temperature and chlorophyll were tested as predictors. However, none of the environmental drivers could explain the microbial composition. Our results indicate that *T. coccinea's* bacterial community may be more influenced by hydrocarbon pollution and colony substratum than by the environmental factors' tested.

Key words: South Atlantic; Latitudinal Gradient; Symbiose; Metabarcoding; 16S rDNA

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Highlights

- The relationship between coral and its microbiota is vital;
- Environmental drivers can modify microbial community structure;
- *Tubastraea coccinea* does not change microbial community structure with latitude;
- Microbial community structure changes significantly in hydrocarbon contaminated zone;

Resumo em linguagem acessível

Recifes de corais estão entre os ecossistemas mais diversos do planeta, e essa diversidade não se limita apenas aos organismos vistos a olho nu. Microrganismos, como bactérias, vírus e fungos são extremamente diversos, abundantes e importantes para a sobrevivência dos corais. No Brasil o coral-sol é uma espécie invasora, e atualmente pode ser encontrado por aproximadamente 3500km da costa. Devido a diversas características oportunistas, o coral-sol vem colocando em risco vários organismos nativos da nossa costa, mudando drasticamente a paisagem marinha. Nosso trabalho teve como objetivo descrever a comunidade de bactérias e arqueias desta espécie invasora, buscando entender se fatores ambientais, como temperatura e nutrientes, de 15 diferentes pontos de coleta estão influenciando a composição e abundância dessa comunidade. Apesar de termos determinado o core microbiano e verificado variações gerais na comunidade microbiana nas amostras de corais em relação as diferentes localidades estudadas, nenhuma variável ambiental verificadas parece explicar a estrutura da comunidade de bactérias do coral-sol da costa brasileira. Apesar disso, há indícios de que áreas contaminadas por óleo e o tipo de substrato podem influenciar essa microbiota.

Bacterial community structure of the coral *Tubastraea coccinea* introduced into the Southwestern Atlantic

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Key words: South Atlantic; Latitudinal Gradient; Symbiose; amplicon metagenomics; 16S rDNA

Introduction

Coral reefs are among the most diverse ecosystems known to mankind [1, 2]. Their main framework builders – corals from the order Scleractinia – have a strong symbiotic relationship with their associated microbiota. Such a relationship is fundamental for the hosts' "health" and, therefore, important for the survival of coral reef ecosystems [3–5]. Since corals do not produce antibodies and are purported to lack an adaptive immune system [6, 7], the microbial community is responsible for their protection and pathogen control [8, 9]. Also, nutrients such as nitrogen and sulfur are recycled and fixed by the microorganisms associated with the coral [4, 10, 11]. Bacteria, archaea, viruses, and microbial eukaryotes belong to this microbial community, of which bacteria appear to be the most numerous, as a single species of coral may be inhabited by more than 1 000 species of bacteria [12, 13]. When environmental conditions change, regulations of the microbiota community allow the holobiont – a term used to refer to the coral host and all organisms associated with it – to adapt more rapidly, increasing chances of survival [14, 15].

The coral microbiome can be divided into two main groups, the core microbiome formed by microorganisms shared between all individuals of the same species, and transient microorganisms [16–18], which could improve fitness by being modified according to the environment. The composition of the core microbiome seems to be species-specific and, in several cases, vertically transmitted [19, 20]. On the other hand, the composition and abundance of transient microorganisms are influenced by several environmental factors [21, 22]. For example, scleractinian corals exposed to high temperatures may have an abundance of bacteria up to 130 times greater than those under normal temperature conditions [23]. Likewise, corals in locations with higher salinity appear to have a greater abundance of bacteria of the genus *Pseudomonas* [13, 24], and changes in turbidity are also recognized as

directly influencing coral microbiota and, therefore, coral survival [25, 26]. Counterintuitively, however, is the fact that the abundance of the coral host at a site influences the diversity and abundance of the bacterial community as well [27].

Overall, the most common bacterial associations in corals are with the classes *Alphaproteobacteria* and *Gammaproteobacteria*, both belonging to the phylum *Proteobacteria* [4, 28, 29]. The bacterial genus *Endozoicomonas* is one of the main members of the communities associated with stony corals [30–32] and may play an important role in the health of these cnidarians. A reduction in its abundance may indicate unfavorable environmental conditions for the host [25, 33, 34].

The azooxanthellate coral *Tubastraea coccinea* Lesson, 1829 (Scleractinia: Dendrophylliidae), popularly known as the orange sun coral, is considered non-native to the Atlantic and invasive along the Brazilian coastline [35]. Its introduction occurred around the 1980s, first recorded on offshore oil platforms in the Campos Basin, Rio de Janeiro [36]. Due to a lack of natural predators, rapid reproduction and extensive defense mechanisms, sun corals have spread rapidly along the Brazilian coastline, threatening native species and modifying ecosystem functions and seascape [37–39]. Currently, *T. coccinea* is found discontinuously along more than 3500 km of the Brazilian coastline (between Ceará and Santa Catarina states), on natural and artificial substrates [40, 41]. On both substrates, Capel et al. [42] indicate that asexual reproduction plays a crucial role in the dynamics of the invasion [40].

Due to its relatively recent introduction in the Southwestern Atlantic, sun corals can be used as model organisms to increase our knowledge of the processes of establishment and adaptation of bacterial communities in early diverging metazoans, with a focus on the composition of the core microbiome and transient microbial community in different locations. Here, based on samples of *T. coccinea* from the whole Southwestern Atlantic range of

51 distribution - from Ceará (2ºS) to Santa Catarina (27ºS) states in Brazil, we describe its
52 microbial symbionts and investigate the influence of environmental factors on transient
53 microbiome composition.

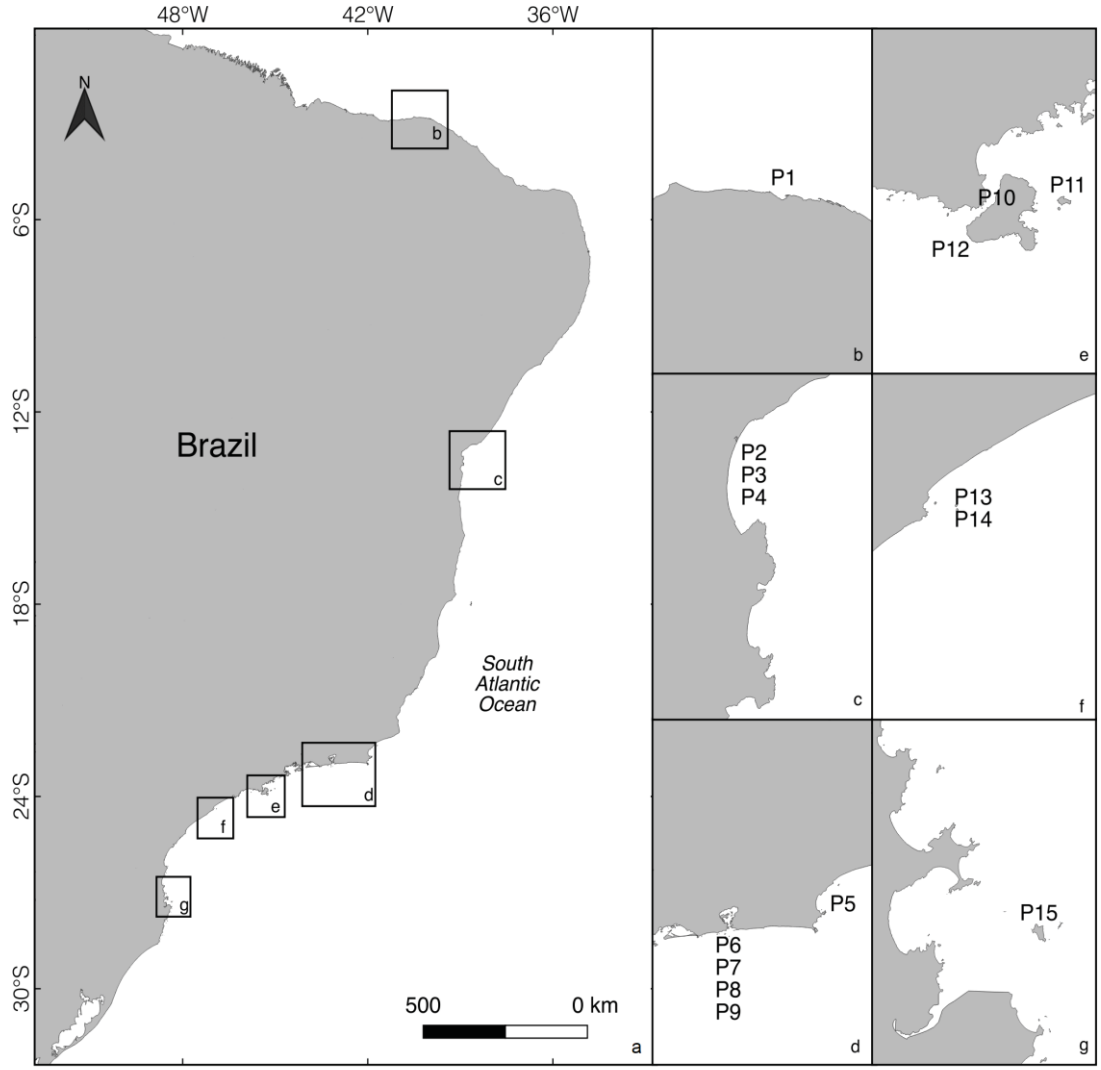
54

55 **Materials and methods**

56

57 *Sampling and study area*

58 *Tubastraea coccinea* colonies were sampled by SCUBA diving at 15 locations
59 spanning 3 500 km of the Brazilian coastline (Figure 1; Table 1). At each location, samples
60 were collected in triplicate and preserved in CHAOS buffer (guanidine thiocyanate 4 M, N-
61 lauryl sarcosil 0.5%, Tris pH 8.0 25 mM, 2-mercaptoethanol 0.1 M) as described by Fukami
62 et al. [43].



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64 **Figure 1** Map with the approximate location of the sampling points. (**a**) Brazilian coast with
65 sampled sites indicated. (**b**) Ceará coast, site P1. (**c**) Todos os Santos Bay, sites P2, P3 and P4.
66 (**d**) Rio de Janeiro, sites P5, P6, P7, P8 and P9. (**e**) São Paulo north coast, sites P10, P11 and
67 P12. (**f**) São Paulo south coast, sites P13 and P14. (**g**) Santa Catarina, site P15.

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72 **Table 1** Sampled site, location, specimen code, longitude (Long), latitude (Lat), and date of
73 collection (month/year).

Site	Location	Samples	Long	Lat	Date
P1	Acaraú Shipwreck	NPA 33; NPA 34; NPA 35	-40.11	-2.55	05/2017
P2	Barra do Paraguaçu	BTS 104; BTS 106; BTS 110	-38.25	-13	03/2013
P3	Itaparica Island	BTS 170; BTS 174; BTS 176	-38.25	-13	07/2012
P4	Cavo Artemidis Wreck	BTS 63; BTS 67; BTS 70	-38.25	-13	10/2012
P5	Ancora Island	IAB 4; IAB 5; IAB 25	-41.8	-22.77	02/2017
P6	Cobras Island	BIG 36; BIG 39; BIG 43	-44.4	-23.08	12/2012
P7	Itaquatiba Island	BIG 187; BIG 188; BIG 192	-44.25	-23.08	07/2017
P8	Bananal	BIG 200; BIG 204; BIG 206	-44.25	-23.08	07/2017
P9	Abraãozinho Beach	BIG 01; BIG 02; BIG 03	-44.15	-23.14	02/2019
P10	Ilhabela	ICI 02; ICI 05; ICI 10	-45	-24	08/2018
P11	Buzios Island	TC 03; TC 07; TC 10	-45	-24	03/2015
P12	Alcatrazes Archipelago	SPAL 21; SPAL 33; SPAL 200	-45.7	-24.08	11/2014
P13	Lajes de Santos	LS 03; LS 04; LS 07	-46.18	-24.29	08/2016
P14	Queimada Grande Island	QC 02; QC 04; QC 05	-46.67	-24.48	08/2016
P15	Arvoredo Island	IA 01; IA 03; IA 06	-48.36	-27.28	11/2018

74

75 The Acaraú shipwreck (P1) is located approximately 30km off the coast of Acaraú
76 city, Ceará State. Mean sea surface temperature (SST) is 27°C [44, 45], with a subtle annual
77 variation (about 1.5°C). Trade winds and coastal currents cause resuspension and transport of

78 marine sediments, increasing seawater turbidity [46], which hinders coral reef formation in
79 the region [47].

80 Barra do Paraguaçu (P2), Itaparica Island (P3), and Cavo Artemidis shipwreck (P4)
81 are located within or nearby Todos os Santos Bay (Bahia State). SST and salinity vary
82 between 24ºC and 30ºC, and 23 and 32.5, respectively, in the rainy and dry periods [48].
83 Coral reefs in the region have undergone significant changes in species composition in recent
84 years, probably due to the increase in the water turbidity caused by pollution and suspended
85 sediments [49].

86 In the north of Rio de Janeiro State, Ancora Island (P5) is influenced by the Cabo Frio
87 upwelling, with annual SST variation of approximately 12ºC (from 13 to 25ºC) [50, 51] and
88 high concentration of nutrients and primary productivity. Further south Cobras Island (P6),
89 Itaquatiba (P7), Bananal (P8) and Abraãozinho (P9) are tropical sites displaying little SST
90 variation throughout the year, ranging between 24ºC and 28ºC in the winter and summer,
91 respectively [52]. This region has a remarkably high abundance of *T. coccinea*, being found in
92 32 of 37 points analyzed by Creed et al. [40, 53].

93 The southeastern Brazilian coast, between 23°05' - 24º05'S, has several localities
94 where *Tubastraea* spp. (*T. coccinea* and *T. tagusensis*) dominate the rocky shores. Located on
95 the sub-tropical northern coast of São Paulo State, Ilhabela (P10), Buzios Island (P11), and
96 Alcatrazes Archipelago (P12) have SST ranging between 21.5 and 25ºC, in winter and
97 summer periods, respectively. Further south, Laje de Santos (P13) and Queimada Grande
98 Island (P14) display overall SST and salinity ranging around 20-27ºC and 34.5-33.5, in the
99 winter and summer respectively [54].

100 The southernmost sampling location (Arvoredo Island, Santa Catarina State, P15)
101 presents a sub-tropical climate with a greater thermal amplitude throughout the year. In the

summer SST is around 27ºC, while in winter, during the direct influence from the discharges of La Plata River and the Falklands Current, it falls to approximately 17ºC [55, 56].

Environmental drivers

SST and chlorophyll-*a* concentration were obtained from the NASA EOSDIS Physical Oceanography Distributed Active Archive Center (PO.DAAC), which comprises SST measured from different satellite sensors [57], and the OceanColor web [58], recorded by the MODIS-Aqua sensor respectively. The nitrate (NO_3), phosphate (PO_4), and iron (Fe) concentrations were obtained from E.U. Copernicus Marine Service which compiles information from both satellite and *in situ* measurements. All data were extracted using the SeaWiFS Data Analysis System (SeaDAS) [59]. Latitude and longitude were also used as environmental driver variables.

DNA extraction, amplification, and sequencing

For the metabarcoding analyses, DNA was extracted from each sample using the DNeasy PowerSoil Kit Qiagen (Germany), following the manufacturer's instructions but using 100 µL of coral-CHAOS solution instead of 0.25g of soil. Libraries preparation followed the Illumina (California, USA) protocol "*16S Metagenomic Sequencing Library Preparation: Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System*", with few modifications. Primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATCTAATCC-3') [60] with Illumina adaptors were used to amplify the V3 and V4 regions of the 16S rDNA gene (~464 bp).

PCR was carried out in 50 µL reaction containing 10 µL of 5X Phusion HF Buffer, 1 µL of 10 mM dNTP mix, 1.75 µL of 10 µM of each primer, 0.5 µL of Phusion High-Fidelity

DNA Polymerase (Thermo Fisher Scientific - Massachusetts, EUA), 2 μL of DNA, and 33 μL of ultra-pure H_2O, using a thermocycling profile of 98°C for 30 s, followed by 30 cycles of 98°C for 10 s, 52°C for 30 s, and 72°C for 20 s. The final extension was performed at 72ºC for 10 min. PCR products were purified with the Agencourt AMPure XP (Beckman Coulter Life Sciences - Indianapolis, USA). To increase amplicon concentration a second PCR was conducted in 25 μL reactions containing the same balance between reagents and thermocycling profile described above but using 1 μL of the previous PCR product as the DNA source. Second-round PCR products were also purified with the Agencourt AMPure XP, and a third PCR was conducted to ligate Illumina dual indices using the Nextera XT Index Kit. The indexing PCR was carried out in 50 μL reaction volume containing 10 μL of 5X Phusion HF Buffer, 1 μL of 10 mM dNTP mix, 0.5 μL of Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific), 5 μL of PCR product, 5 μL of Index N7xx, 5 μL of Index S5xx, and 23.50 μL of ultra-pure H_2O, using the thermocycling profile of 98°C for 30 s, followed by 8 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 20 s, and finally 72ºC for 10 min. The Index PCR products were also purified using the Agencourt AMPure XP beads. Libraries were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific), pooled at 2nM, and paired-end sequenced with the 600 cycles MiSeq (Illumina) reagent kit V3, with 20% of PhiX. Sequencing was performed at the Genome Investigation and Analysis Laboratory at Core Facility for Scientific Research – University of São Paulo (CEFAP-USP/ GENIAL).

Bioinformatics

Low-quality and short DNA sequences (<50bp) were removed using SolexaQA++ [61], and pair-end reads were assembled with PANDAseq [62]. Exact copies were dereplicated, and the Swarm software [63] was used to cluster identical sequences into OTUs.

151 Sequences were then classified at the genus level on the Mothur platform [64] using the 16S
152 Silva-v.132 database [65].

153 Statistical analyses were performed using the computing environment *R* [66]. *WGCNA*
154 package was used to generate the microbial structure hierarchical cluster analyses [67].
155 Packages *reshape*, *ggplot2* and *scales* were applied to produce the phyla and classes relative
156 abundance plots [68, 69]. Shannon Diversity Index was calculated using the *vegan* package,
157 and the respective plot was performed by the *barplot* function [70].

158 Principal Components Analysis (PCA) performed at *FactoMineR* was used to
159 visualize the similarity among samples [71]. To identify co-variation groups and relate them
160 to the environmental variables, a Weighted Gene Co-expression Network Analysis
161 (WGCNA), was performed, using the *WGCNA* package. In the WGCNA, rare genera – those
162 with abundance equal to or smaller than 2 in any analyzed sample – were removed. SoftPower
163 was defined as 6, and the minimum module size was defined as 5.

164

Results

Sequencing Output and Core Microbiome

A total of 24 287 168 reads from the 45 *T. coccinea* samples were used for Operational Taxonomic Units (OTUs) prediction, resulting in 2 660 genera, 817 families, 450 orders, 170 classes, and 65 phyla of *Bacteria* and *Archaea*. The number of bacterial OTUs in each sample was significantly higher, varying from 114 to 1 499, probably due to the specificity of the primers. Excluding eukaryotes, chloroplast and mitochondrial sequences, *Bacteria* accounted for 99.5% of all sequences retrieved (Supplementary material 1). Due to the outlier values of reads and OTUs, samples BTS 170, BTS 174, and BTS 176 were excluded from further analyses. A total of 215 *Bacteria* and *Archaea* genera (OTUs) found in all 42 samples were defined as the *T. coccinea* core microbiome (Supplementary material 2).

Diversity and Environmental factors

Overall, the microbial community composition of *Tubastraea coccinea* along the Southwestern Atlantic could be divided into two groups (Figure 2a). Ilhabela's samples were the most dissimilar, but, in general, samples from the same location were highly similar in terms of composition. Interestingly, the microbial community from Acaraú shipwreck and Queimada Grande Island presented high similarity, even though these localities are latitudinally distant (~22º).

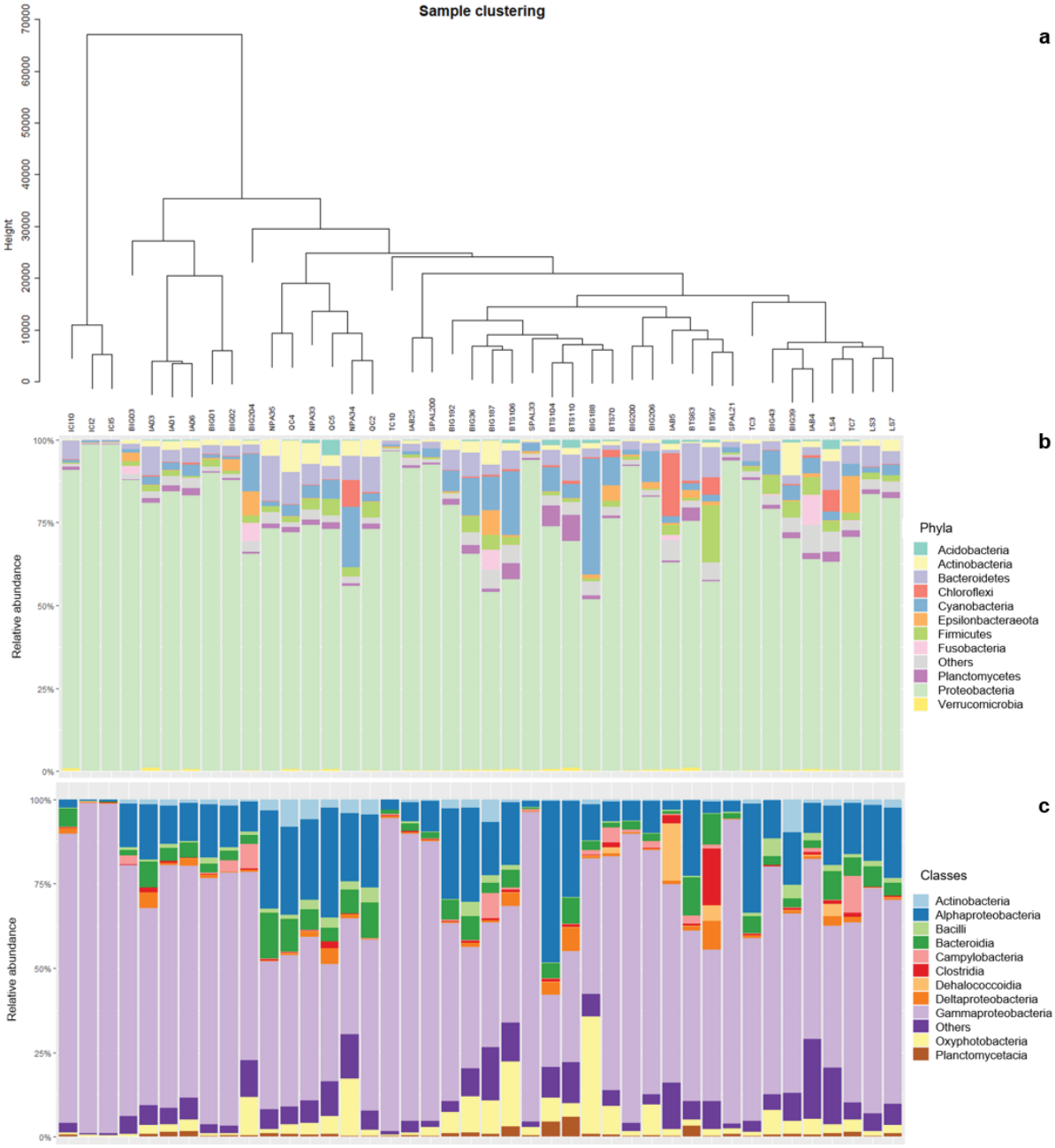

Figure 2 Sample clustering and relative abundance of the most abundant phyla and classes from the 42 analyzed samples. (**a**) Hierarchical cluster analysis (method = "average") according to microbial community composition. (**b**) Relative abundance of the eleven most abundant phyla in each sample. (**c**) Relative abundance of the eleven most abundant classes in each sample.

The most abundant phylum was *Proteobacteria* (77.95% of all samples), followed by *Bacteroidetes* (5.14%), *Cyanobacteria* (4.24%), *Actinobacteria* (2.46%) and *Firmicutes* (2.31%). *Proteobacteria* was also the most abundant phylum in each sample, with a relative abundance higher than 50% in all samples (Figure 2b). At the class level, the most abundant were *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacteroidia*, *Oxyphotobacteria,* and *Actinobacteria* (Figure 2c).

Ilhabela samples presented the lowest diversity (2.1 ± 0.5), while those from Barra do Paraguaçu presented the highest diversity (5.6 ± 0.2) (Figure 3). Except for Ilhabela and SPAL 33 (Alcatrazes Archipelago), all samples presented a Shannon index above 3, indicating high microbial diversity.

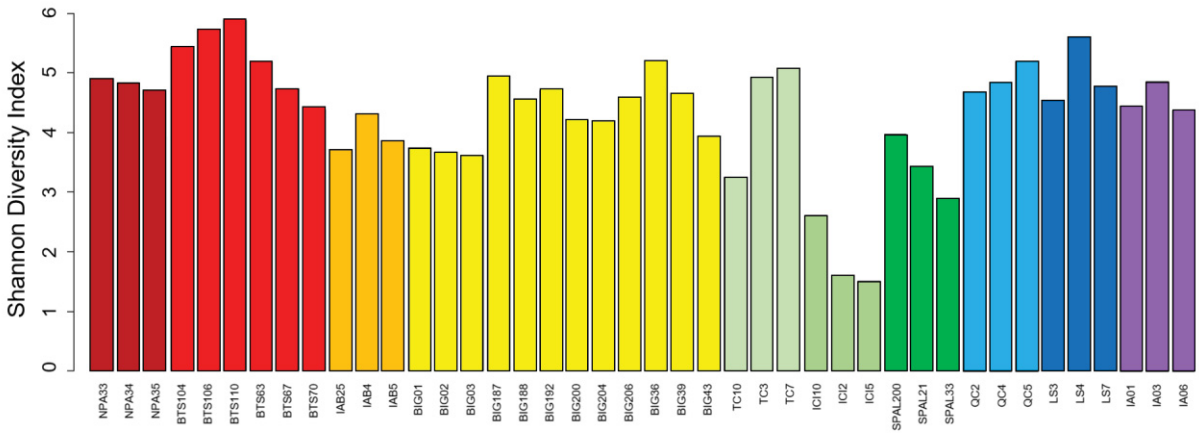

Figure 3 Shannon Diversity Index from the 42 samples of the microbiota of *Tubastraea coccinea* collected along the Brazilian coast. The bars are colored according to the sample region, being darker red closer to Equator, while purple are the southern.

The correlation of the microbial community from each sample with environmental factors resulted in a different clustering pattern than that retrieved using microbial community composition (Supplementary material 3). This suggests that the environmental factors tested

herein could not explain the microbial composition from different environmental conditions. Nevertheless, environmental conditions could influence the abundance of co-varying groups. The WGCNA identified six modules of co-variation which were named as colors (Figure 4), of which the blue group consisted of 20 genera, brown group of 8 genera, green group of 5 genera, gray group of 28 genera, turquoise group of 29 genera, and yellow group of 8 genera (Supplementary material 4). Nitrate (NO_3) and phosphate (PO_4) were the environmental variables with the strongest negative relation to the groups, especially for the turquoise and blue groups. Latitude and chlorophyll-a had the highest positive relationship with the turquoise group.

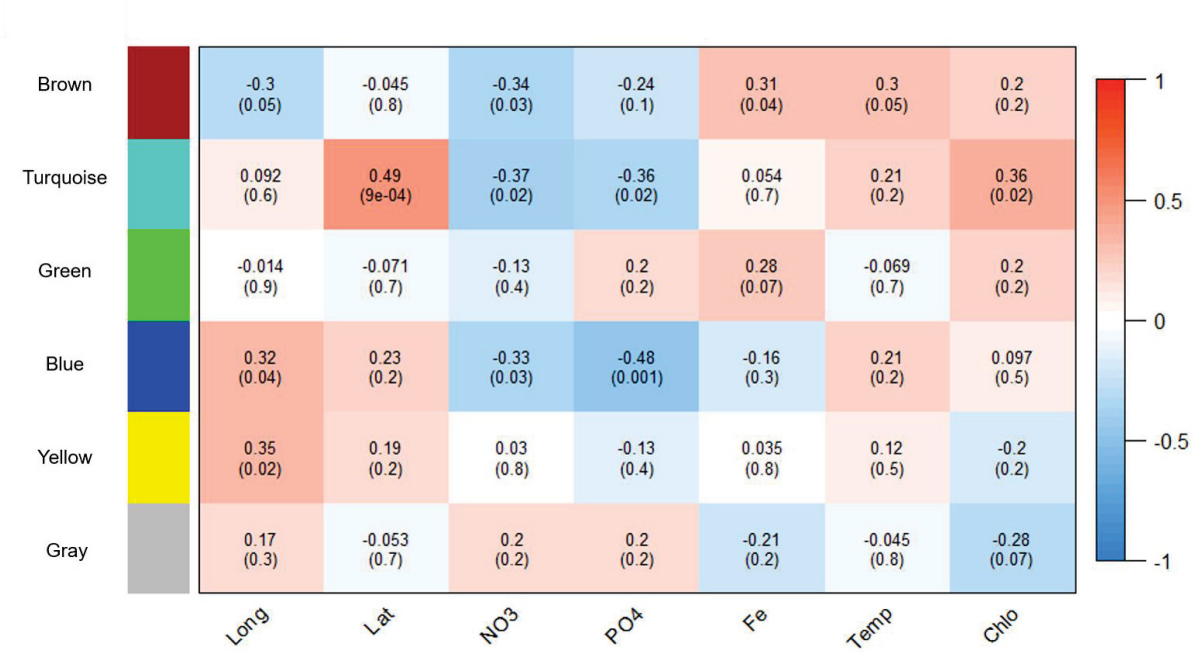

Figure 4 Weighted correlation network analysis (WGCNA) of *T. coccinea* microbial community. Each color represents a co-variation group. The intensity of red and blue coloring indicates the strength of the positive and negative correlation respectively (correlation value could vary between 1 and -1). Both, red and blue, represent the correlation between groups and environmental factors. The value of these correlations and its *p-value* (in parentheses) are indicated inside each box. Long = Longitude; Lat = Latitude; NO_3 = Nitrate; PO_4 = Phosphate; Fe = Iron; Temp = Temperature (ºC); and Chlo = chlorophyll-*a*.

Discussion

Sequencing Output and Core Microbiome

Corals are among the most diverse meta-organisms, with the number of microbial OTUs ranging up to 10^2–10^4 and a substantial variation between host species [72]. Our results demonstrated that *T. coccinea* from Southwestern Atlantic has, on average, around 920 OTUs, a value higher than previously found for the same species from Caribbean [73], but similar to that found by Carlos et al.[20] also in Southwestern Atlantic. As sequencing methods and primer selection greatly influence the contribution to the number of OTUs found in coral microbiomes [74] the comparison of results derived from different sequencing platforms and primers is challenging or even unrealistic. Furthermore, although the definition of core microbiome is still under debate [74], we adopted that "all OTUs present in all samples" should be species-specific. The classes *Actinomycelates* and *Burkholderiales* have been suggested as the two most abundant groups in a "universal coral core microbiome" [16, 75]. However, although representatives of *Burkholderiales* were present abundantly in all analyzed samples, *Actinomycelates* were not found in all samples and had low abundance when present. It is important to note that the suggested "universal coral core microbiome" was based on zooxanthellate coral species [16]. Therefore, it might not apply to all coral species, especially the azooxanthellate, such as those present here. It is interesting that the core microbiome from the deep-sea azooxanthellate coral *Lophelia pertusa* did not present *Burkholderiales* [76], and the one from *Eguchipsammia fistula*, another deep-sea azooxanthellate species, seems to be composed of only six bacterial taxa, none of them belonging to *Actinomycelates* or *Burkholderiales* [77]. One reason for this disparity in the microbial community among azooxanthellate taxa could be due to depth differences. *Tubastraea coccinea*, although azooxanthellate, is a shallow water species, while *L. pertusa*

and *E. fistula* occurs below 100 meters depth. These results may reflect not only how complex coral microbiomes are, but also how challenging it is to define a universal coral microbiome core for both "ecological groups".

Diversity and Environmental factors

In the marine realm, 18 of the 20 most abundant *Bacteria* belong to the phylum *Proteobacteria* [78]. As previously found for other stony corals [12, 13], including sun corals [20], here we also recovered a high proportion – higher than 75% – of *Proteobacteria* in the *T. coccinea* microbiome (Figure 2b), even though the Shannon diversity index indicated a highly diverse microbial community. Similar proportions of *Proteobacteria* have previously been found in populations of invasive sun corals in the Atlantic (Caribbean and Southwestern Atlantic) [20, 73]. At the class level, *Gammaproteobacteria* was significantly more abundant than other classes, contrasting to native *T. coccinea* from East China, [79] from which *Betaproteobacteria* had a higher abundance. Such differences may indicate that the microbiome from the Atlantic invasive *T. coccinea* has adapted to the new environment.

Despite none of the environmental factors analyzed could explain the microbiome of *T. coccinea* throughout the geographical range analyzed, characteristics such as pollution and substrate seem to influence their microbial community. Samples from Ilhabela were collected inside a marina breakwater known to be chronically impacted by hydrocarbons (*personal observation*) and were the most dissimilar and with the lowest diversity values when compared to all other (Figures 2a and 3). The particular conditions of this site create a very unique environment compared to all other sampled sites, which is probably challenging to the survival of most microorganisms, leading to a less diverse and more specific microbial community. The *Gammaproteobacteria* class was recovered with a very high relative

abundance (>90%, Figure 3c) for Ilhabela samples ICI 2 and ICI 5, and previous studies have demonstrated that *Gammaproteobacteria* abundance tends to increase in the presence of hydrocarbons [80, 81]. At the genus level, *Endozoicomonas* was the most abundant for all Ilhabela samples (Supplementary material 5). This *Gammaproteobacteria* genus is commonly found in marine invertebrates and is suggested to be positively associated with healthy individuals, particularly in corals [25, 33, 34]. The occurrence of high *Endozoicomonas* abundance in these samples is, therefore, counterintuitive. That being said, studies with *Endozoicomonas* indicate that even though commonly associated with health organisms, this genus performs several functions in protein and carbohydrate transport and cycling [34]. The genus also harbors a high degree of genomic plasticity, allowing rapid adaptation, indicating that different genotypes may play different roles in their hosts [82]. This capability of rapid adaptation may allow *Endozoicomonas* to better resist polluted environments compared to other bacterial groups, leading to their higher abundance.

Apart from pollutants, as sessile invertebrates, the substrate is recognized to affect coral larvae settlement [83, 84]. Besides the physical properties of the substrate, its biofilm is also thought to play a key role in the induction of larval settlement and metamorphosis [85]. However, although it is still unclear if the biofilm influences the coral microbiome, our results suggested that it may occur. *Tubastraea coccinea* colonies from Acaraú and Queimada Grande Island were both sampled attached to shipwreck hulls and, although being under different environmental conditions and apart from more than 3 000 km, displayed a very similar microbial structure (Figure 2a). Although it is still unclear if the substrate biofilm influences the coral microbiome, our results suggested that this may occur. Consequently, the type of substrate, such as steel, may “guide” or “interact” significantly with the bacterial community structure from *T. coccinea*.

The influence of environmental factors on corals and also on its endobacteria have been widely studied [21–24]. However, none of the environmental factors analyzed here explained the differences in the bacterial community of *T. coccinea*. A recent study on how the bacterial community responds to cross-transplantation found that the bacterial community from *Acropora hemprichii* was affected by different levels of anthropogenic impact, while that from *Pocillopora verrucosa* remained stable [86]. The authors suggested that the degree of microbiome flexibility may be linked to the life history traits of the host. While *A. hemprichii* has a longer generation time *P. verrucosa* favors an opportunistic colonization strategy, characterized by fast growth, high reproduction rates, and relatively rapid generation times, similar to *T. coccinea* [87]. Also, populations of invasive *T. coccinea* in the Southwestern Atlantic are known to have high levels of clonality derived from asexual reproduction [42]. Due to its life strategy and low genetic diversity, *T. coccinea* may be able to maintain a relatively stable bacterial community across a large latitudinal gradient. However, it is still unknown if the microbiome flexibility can shape the life history strategy or otherwise.

Moreover, corroborating a co-evolutionary path between host and microbiota, "geography" has been thought to have a trifling influence on the microbiota of *Pocillopora damicornis* and, although temperature affected the eukaryote symbionts of the coral (i.e. *Symbiodinium* – commonly known as zooxanthella), it did not significantly alter the coral endobacterial [31]. Thus, different groups of endosymbionts respond differently to changes in environmental conditions. Herein, even that there was not a clear relationship between the bacterial community structure from *T. coccinea* to the tested environmental factors (i.e. temperature, nitrate, phosphate, iron and chlorophyll-*a*), we cannot assume that those features did not influence the whole microbial community, like viruses, fungus, and other microeukaryotes were not studied. Overall, when coral symbionts other than zooxanthellae

are analyzed, it is still unclear which environmental factors (or their synergetic effects) mostly affects them. Also, as bacterial communities from different coral species (or even populations) may respond distinctively to environmental changes predictions are nothing less than challenging.

The WGCNA heatmap indicates that nitrate and phosphate have a mainly negative influence with the co-variation groups (Figure 4). The blue group is particularly negatively-affected by phosphate concentration; high concentrations of these nutrients are indicative of poorer water quality and have been shown to negatively affect coral health [88]. Microorganisms from the largest, group (turquoise) increased their abundance at lower latitudes. As lower latitudes are expected to present higher temperatures, it was expected that the turquoise group would also show a strong positive relationship with temperature. Nevertheless, such a relationship was not statistically significant (*p-value*: 0.2). In fact, none of the correlations of the WGCNA heatmap was higher than 0.49, or lower than -0.48, and most of them are not statistically significant. Even though satellite and reanalyses (compiled information from both satellite and *in situ* observations data) are a useful source of information, it is important to stress that environmental data from *in situ* measurements would probably have resulted in more meaningful, stronger correlations. Critical processes occurring at a finer scale than the pixel area of the satellite data are usually masked and result in information loss. The Brazilian coastal zone is a complex and dynamic environment, displaying fine scale but intense physical-chemical-biological processes. Therefore, using satellite data may have reduced our sensitivity in interpreting important environmental factors acting on the microbiome from *T. coccinea*.

Corals' microbiomes are diverse, complex, and variable both inter and intraspecifically. A better understanding of how the microbiome would respond to environmental drivers is crucial to predict how the host might respond to local (e.g. pollution)

352 or regional/global (e.g. warming climate) anthropogenic challenges. Our results suggest that
353 the microbial community structure of *T. coccinea* does not vary within a gradient of latitude,
354 temperature, or nutrients. Further studies testing other environmental conditions, such as
355 turbidity, substrate, and water contaminants could provide a better picture of how the
356 microbial community from *T. coccinea* is shaped through space and time.

357

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590

Supplementary material 1

Number of sequences retrieved per sample. Total number of Operational Taxonomic Units (OTUs), and number of OTUs, at genus level from Eukaryote, Bacteria and Archaea.

Sample Name		Number of Sequences	Sequences longer than 50 bp	Identified Sequences	Sequences of Bacteria and Archaea	OTUs Total	OTUs Eukaryota	OTUs Bacteria	OTUs Archaea
BIG01	R1	237647	237647	203616	149636	1114	238	848	28
	R2	237647	237024						
BIG02	R1	206284	205716	178381	134183	1012	194	789	29
	R2	206284	205728						
BIG03	R1	237390	237086	207930	166711	1011	189	796	26
	R2	237390	237092						
BIG36	R1	252248	251797	212008	99466	1210	206	974	30
	R2	252248	251779						
BIG39	R1	308830	308469	250965	87560	1278	270	974	34
	R2	308830	308472						
BIG43	R1	280881	280533	233898	89811	983	243	714	26
	R2	280881	280543						
BIG187	R1	221567	221371	176683	81558	1158	202	932	24
	R2	221567	221381						
BIG188	R1	163355	163278	127200	32942	918	204	694	20
	R2	163355	163283						
BIG192	R1	287303	287110	236781	116852	1234	200	1004	30
	R2	287303	287118						
BIG200	R1	379445	378959	319888	149580	1100	208	869	23
	R2	379445	378961						
BIG204	R1	270268	270128	229833	108752	1152	190	933	29
	R2	270268	270121						
BIG206	R1	304975	304765	263493	128252	1114	185	902	27

	R2	304975	304776						
BTS63	R1	424909	418155	361443	204240	1452	262	1156	34
	R2	424909	418093						
BTS67	R1	318747	318643	277946	162043	1338	178	1129	31
	R2	318747	318636						
BTS70	R1	155187	155162	126257	56129	1017	160	831	26
	R2	155187	155155						
BTS104	R1	303243	303050	245848	170990	1665	204	1424	37
	R2	303243	303063						
BTS106	R1	341797	341530	271773	113170	1596	259	1296	41
	R2	341797	341569						
BTS110	R1	298277	298153	242437	152304	1706	173	1499	34
	R2	298277	298277						
BTS170	R1	1153	1153	689	285	163	22	137	4
	R2	1153	1152						
BTS174	R1	572	572	349	234	120	6	114	0
	R2	572	572						
BTS176	R1	1334	1330	843	576	213	21	191	1
	R2	1334	1329						
IA01	R1	217448	216904	178203	107283	1297	211	1053	33
	R2	217448	216911						
IA03	R1	211500	211056	177320	119723	1310	166	1112	32
	R2	211500	211075						
IA06	R1	264614	264390	216005	96401	1336	193	1115	28
	R2	264614	264405						
IAB4	R1	402790	402488	309435	79123	1202	290	880	32
	R2	402790	402488						
IAB5	R1	245176	245064	204269	124544	1105	195	878	32
	R2	245176	245054						
IAB25	R1	135629	135555	106152	51860	968	179	767	22

	R2	135629	135547						
ICI2	R1	259116	259037	215034	170307	792	127	644	21
	R2	259116	259045						
ICI5	R1	241712	241686	209247	176373	898	105	770	23
	R2	241712	241692						
ICI10	R1	259696	259660	225127	213093	1317	105	1187	25
	R2	259696	259656						
LS3	R1	375630	374741	295772	109076	1169	308	831	30
	R2	375630	374798						
LS4	R1	375268	374885	305666	132236	1470	263	1168	39
	R2	375268	374909						
LS7	R1	338457	338122	265460	92131	1214	276	908	30
	R2	338457	338120						
NPA33	R1	316177	314684	277106	203322	1230	261	937	32
	R2	316177	314634						
NPA34	R1	362970	362315	316110	193462	1213	250	934	29
	R2	362970	362309						
NPA35	R1	299057	297450	269119	241782	1184	294	859	31
	R2	299057	297468						
QC2	R1	343421	342280	274671	131760	1104	267	808	29
	R2	343421	342362						
QC4	R1	253851	251327	222830	207278	1112	231	852	29
	R2	253851	251419						
QC5	R1	253063	252524	215553	167943	1256	230	997	29
	R2	253063	252588						
SPAL21	R1	290159	289891	245868	110524	1009	164	823	22
	R2	290159	289881						
SPAL33	R1	336266	336186	36094	15471	534	76	449	9
	R2	336266	336253						
SPAL200	R1	286118	286030	244611	110518	1029	181	821	27

	R2	286118	286028						
	R1	383452	383154						
TC3				307131	139314	1302	225	1047	30
	R2	383452	383156						
	R1	316407	316234						
TC7				254887	112268	1371	253	1087	31
	R2	316407	316238						
	R1	380195	380025						
TC10				333647	275065	1182	183	971	28
	R2	380195	380039						

Supplementary material 2

All genera identified from all 42 samples and classified as the *Tubastraea coccinea* microbial core.

ABY1_ge, Acetobacterales_Incertae_Sedis_ge, Acidiferrobacteraceae_ge, Acidobacteria_ge, Aerosakkonema_Lao26, Aestuariispira, Aestuariivivens, Alkalispirillum, Allobacillus, Alphaproteobacteria_ge, Alpinimonas, Alteromonadaceae_ge, Alteromonadales_ge, Amoebophilaceae_ge, Ampullimonas, Anabaena_XPORK15F, Anaplasmataceae_un, Aquabacter, Aquincola, Arenicellaceae_ge, Arenitalea, ATCC-39006, Atelocyanobacterium_(UCYN-A), Auraticoccus, AUTHM297, Azomonas, Azorhizophilus, B29_ge, Bacillales_ge, Balneatrix, Basilea, Betaproteobacteriales_ge, Betaproteobacteriales_Incertae_Sedis_ge, Brackiella, Brocadiales_ge, Calderihabitans, Caldivirga, Calothrix_336-3, Candidatus_Allobeggiatoa, Candidatus_Cryptoprodotis, Candidatus_Defluviella, Candidatus_Desulfamplus, Candidatus_Doudnabacteria_ge, Candidatus_Fritschea, Candidatus_Hartigia, Candidatus_Ishikawaella, Candidatus_Kleidoceria, Candidatus_Maribeggiatoa, Candidatus_Nanosalinarum, Candidatus_Nitrosoglobus, Candidatus_Paenicardinium, Candidatus_Paraholospora, Candidatus_Photodesmus, Candidatus_Purcelliella, Candidatus_Rubidus, Candidatus_Westeberhardia, Candidatus_Zinderia, CAP-aah99b04_ge, Caulobacteraceae_ge, Caulobacteraceae_un, Chitinibacteraceae_ge, Chlamydiaceae_ge, Chroococcus_HUW_799, Clade_I_ge, Coleofasciculaceae_ge, Colwelliaceae_ge, Corticibacterium, Crenotalea, Criblamydia, Criblamydiaceae_un, Cricetibacter, Crocinitomicaceae_ge, Crocosphaera_WH_0003_(UCYN-B), Cryomorphaceae_ge, Cylindrospermum_NQAIF308, Cylindrospermum_SAG_11.82, Cytophagales_ge, Cytophagales_un_ge, Daeguia, Deferribacteraceae_ge, Deinococcaceae_ge,

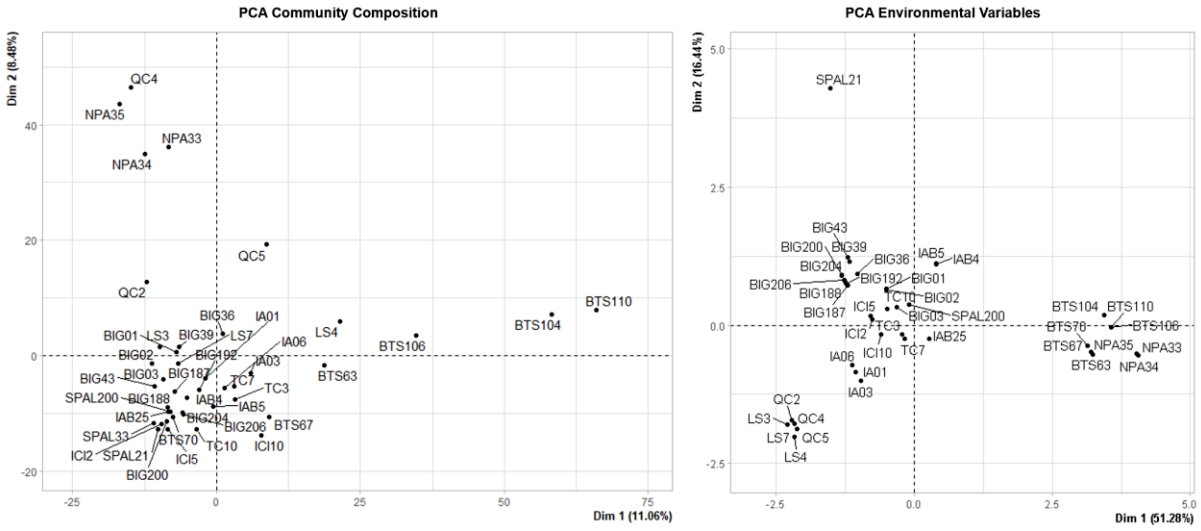
Desantisbacteria_ge, Desulfacinum, Ectothiorhodosinus, Eggerthia, Eionea, EMP-G18_ge, Enterobacteriaceae_ge11, Enterobacteriaceae_ge6, Family_XI_ge, Fangia, FFCH5858, Fischerbacteria_ge, Flavobacteriales_ge, Flavobacterium, Foliisarcina_CENA333, Fortiea_HA4221-MV2, Frederiksenia, Fretibacter, Frischella, GAS113, Gayadomonas, GB102_ge, GBS-1_ge, Geitlerinema_LD9, Gulbenkiania, Gulosibacter, Haloleptolyngbya_KR2005-106, Halorhodospiraceae_un, HdN1, Helicobacteraceae_ge, Hephaestia, Homoserinimonas, Humitalea, IheB2-31, Imtechella, Jiella, JL-ETNP-Z34, Kallotenue, Kineosporiaceae_ge, Kinneretia, Koukoulia, Leeia, Leminorella, Litoribrevibacter, Litorisediminicola, Litorisediminivivens, Lutaonella, Lysobacter, Magnetospiraceae_ge, Maliponia, MBIC10086, Merismopedia_AICB1015, Mesoaciditogaceae_un, Methanobacteriaceae_un, Methylogaea, Methyloligellaceae_ge, Methylomarinovum, Methylophagaceae_ge, Methylophilaceae_ge, Methylopilaceae_ge, Methylosoma, MIZ36, Motilimonas, Neiella, Neisseriaceae_ge, Neosynechococcus, Nibrella, Nitrincolaceae_ge, Niveitalea, Nostocales_ge, Oceanibacterium, Oceanicoccus, P6-b51_ge, Parablastomonas, Paracaedibacteraceae_ge, Paraherbaspirillum, Parvibaculaceae_ge, Pelobium, Pelomonas, Phaeochromatium, Phormidium_ETS-05, Phormidium_MBIC10002, Phormidium_SAG_81.79, Phycisphaeraceae_ge, Pleionea, PMMR1, Postechiella, Proteobacteria_ge, Pseudanabaena_NgrPSln22, Pseudanabaenaceae_ge, Pseudoalteromonadaceae_un, Pseudoroseicyclus, Puniceicoccaceae_ge, Puniceispirillales_ge, Qingshengfania, Ralstonia, S15-21_ge, Saccharospirillaceae_ge, Salinibacillus, SBYZ-1017_ge, SBZC-1223, SD04E11, SM23-32_ge, SN8, Spirillum, Spongiibacteraceae_ge, Spongiivirga, SS1-B-07-19_ge, Stenotrophomonas, Stigonema_SAG_48.90, Sulfurisphaera, Synechococcus_MBIC10613, Synechococcus_PCC-6312, Synechococcus_PCC-7902, Terasakiellaceae_ge, Thermodesulfobacteriaceae_ge, Thermoplasmataceae_ge, Thermoproteaceae_ge, Thermorudis, Thermostilla,

644 *Thioalkalibacter, Thioalkalicoccus, Thiohalobacter, Thiophaeococcus, Thioploca,*
645 *Thiotrichaceae_ge, Thorsellia, Verrucomicrobiales_ge, Vibrionaceae_ge,*
646 *Xanthomonadaceae_ge, Xylanibacterium, Yimella, Z195MB87, Zhizhongheella.*

647

Supplementary material 3

Principal Coordinate Analysis (PCA) representing the similarities/dissimilarities of microbial community composition (a) and environmental variables (b).



Supplementary material 4

Genera belonging to each color block in the Weighted Gene Co-expression Network Analysis (WGCNA).

Brown: *Kinneretia, Pelomonas, Gulbenkiania, Aquincola, Zhizhongheella, Basilea, Neisseriaceae_ge, Koukoulia*

Turquoise: *Caulobacteraceae_ge, Xanthomonadaceae_ge, Caulobacteraceae_un, Paraherbaspirillum, Hephaestia, Ralstonia, Stenotrophomonas, SN8, Parablastomonas, Leminorella, Nibrella, Corticibacterium, Qingshengfania, Azorhizophilus, Candidatus_Doudnabacteria_ge, Candidatus_Rubidus, Methyloligellaceae_ge, Methylogaea, Mesoaciditogaceae_un, S15-21_ge, Z195MB87, Acidiferrobacteraceae_ge, Candidatus_Allobeggiatoa, Candidatus_Nanosalinarum, Crenotalea, HdN1, Balneatrix, Candidatus_Paraholospora, ABY1_ge*

Green: *Candidatus_Zinderia, Methanobacteriaceae_un, B29_ge, Criblamydia, SBYZ-1017_ge*

Blue: *Thermostilla, Spongiibacteraceae_ge, Alkalispirillum, Magnetospiraceae_ge, Arenicellaceae_ge, Phycisphaeraceae_ge, Thiohalobacter, Ectothiorhodosinus, Litorisediminicola, Phaeochromatium, Halorhodospiraceae_un, Aestuariispira, GB102_ge, JL-ETNP-Z34, Puniceicoccaceae_ge, IheB2-31, Alteromonadales_ge, Spongiivirga, Candidatus_Nitrosoglobus, Thiotrichaceae_ge*

Yellow: *Synechococcus_MBIC10613, MIZ36, Pseudanabaena_NgrPSln22, Crocosphaera_WH_0003_(UCYN-B), Foliisarcina_CENA333, Synechococcus_PCC-6312, Coleofasciculaceae_ge, Haloleptolyngbya_KR2005-106*

Grey: *Litoribrevibacter, Nitrincolaceae_ge, Vibrionaceae_ge, Methylophagaceae_ge, Neiella, Eionea, Motilimonas, Pseudoalteromonadaceae_un, Colwelliaceae_ge,*

677 *Gayadomonas, SS1-B-07-19_ge, Azomonas, Oceanicoccus, P6-b51_ge, Pleionea,*
678 *Aerosakkonema_Lao26, endosymbionts6, Neosynechococcus, Flavobacteriales_ge, ATCC-*
679 *39006, endosymbionts11, Methylosoma, Candidatus_Hartigia, Terasakiellaceae_ge,*
680 *Candidatus_Purcelliella, Candidatus_Ishikawaella, Chlamydiaceae_ge,*
681 *Candidatus_Westeberhardia*

682

683 **Supplementary material 5**

684 The five most abundant genus-level OTUs from each analyzed sample.

Sample	Genus	Sample	Genus	Sample	Genus
BIG01	*Kinneretia* *Pelomonas* *Roseibium* *Candidatus_Zinderia* *Gulbenkiania*	BIG02	*Kinneretia* *Pelomonas* *Candidatus_Zinderia* *Gulbenkiania* *Roseibium*	BIG03	*Kinneretia* *Methylophagaceae_ge* *Pelomonas* *Marine_Methylotrophic_Group_3* *Roseibium*
BIG36	*Candidatus_Zinderia* *Caulobacteraceae_ge* *Thalassobaculales_un* *Xanthomonadaceae_ge* *Kinneretia*	BIG39	*Candidatus_Zinderia* *Kinneretia* *Caulobacteraceae_ge* *Xanthomonadaceae_ge* *Paraherbaspirillum*	BIG43	*Candidatus_Zinderia* *Catenococcus* *Vibrionaceae_ge* *Kinneretia* *Photobacterium*
BIG187	*Candidatus_Zinderia* *Fusobacteriaceae_ge* *Helicobacteraceae_ge* *Nitrincolaceae_ge* *Litoribrevibacter*	BIG188	*Anabaena_XPORK15F* *Candidatus_Zinderia* *Neosynechococcus* *Leptolyngbyaceae_MIZ36* *Vibrionaceae_ge*	BIG192	*Candidatus_Zinderia* *Nitrincolaceae_ge* *Endozoicomonas* *Litoribrevibacter* *Polymorphum*
BIG200	*Pseudoalteromonas* *Pseudoalteromonadaceae_un* *Catenococcus* *Candidatus_Zinderia* *Vibrionaceae_ge*	BIG204	*Neiella* *Candidatus_Zinderia* *Helicobacteraceae_ge* *Fusobacteriaceae_ge* *Synechococcus_CC9902*	BIG206	*Catenococcus* *Vibrionaceae_ge* *Vibrio* *Candidatus_Zinderia* *Pseudoalteromonadaceae_un*
BTS63	*Catenococcus* *Vibrionaceae_ge* *Candidatus_Zinderia* *Nitrincolaceae_ge* *Kordiimonas*	BTS67	*Catenococcus* *Vibrionaceae_ge* *Fusibacter* *Dehalococcoidia_SAR202_clade_ge* *Clostridiaceae_4_un*	BTS70	*Catenococcus* *Vibrionaceae_ge* *Neiella* *Candidatus_Zinderia* *Vibrio*
BTS104	*Citreimonas* *Cribrihabitans* *Ruegeria* *Litorisediminivivens* *Aestuariihabitans*	BTS106	*Chroococcus_HUW_799* *Candidatus_Zinderia* *Vibrionaceae_ge* *Synechococcus_MBIC10613* *Synechococcus_CC9902*	BTS110	*Vibrionaceae_ge* *Candidatus_Zinderia* *Thermostilla* *Cribrihabitans* *Catenococcus*
IA01	*Kinneretia* *Pelomonas* *Candidatus_Zinderia* *Gulbenkiania* *Zhizhongheella*	IA03	*Kinneretia* *Pelomonas* *Candidatus_Zinderia* *Gulbenkiania* *Zhizhongheella*	IA06	*Kinneretia* *Pelomonas* *Candidatus_Zinderia* *Gulbenkiania* *Zhizhongheella*
IAB4	*Candidatus_Zinderia* *Fusobacteriaceae_ge* *Kinneretia* *Anaeromusa* *Caulobacteraceae_ge*	IAB5	*Dehalococcoidia_SAR202_clade_ge* *Vibrio* *Vibrionaceae_ge* *Candidatus_Zinderia* *Motilimonas*	IAB25	*Methylophagaceae_ge* *Candidatus_Zinderia* *Marine_Methylotrophic_Group_3* *Candidatus_Endoecteinascidia* *Neiella*
ICI2	*Endozoicomonas* *Litoribrevibacter* *Nitrincolaceae_ge* *Candidatus_Zinderia* *Eionea*	ICI5	*Endozoicomonas* *Litoribrevibacter* *Nitrincolaceae_ge* *Candidatus_Zinderia* *Cellvibrionaceae_ge*	ICI10	*Endozoicomonas* *Litoribrevibacter* *Nitrincolaceae_ge* *Eionea* *Cellvibrionaceae_ge*

	Candidatus_Zinderia		*Candidatus_Zinderia*		*Candidatus_Zinderia*
	Alteromonadaceae_XY-R5		*Eionea*		*Kinneretia*
LS3	*Kinneretia*	LS4	*SAR202_clade_ge*	LS7	*Allofrancisella*
	Aestuariibacter		*Kinneretia*		*Caulobacteraceae_ge*
	Stenotrophomonas		*Caulobacteraceae_ge*		*Caulobacteraceae_un*
	Kinneretia		*Oxyphotobacteria_CENA359*		*Kinneretia*
	Caulobacteraceae_ge		*Candidatus_Zinderia*		*Xanthomonadaceae_ge*
NPA33	*Xanthomonadaceae_ge*	NPA34	*Kinneretia*	NPA35	*Caulobacteraceae_ge*
	Candidatus_Zinderia		*Xanthomonadaceae_ge*		*Hephaestia*
	Caulobacteraceae_un		*Caulobacteraceae_ge*		*Caulobacteraceae_un*
	Candidatus_Zinderia		*Kinneretia*		*Caulobacteraceae_ge*
	Caulobacteraceae_ge		*Xanthomonadaceae_ge*		*Caulobacteraceae_un*
QC2	*Kinneretia*	QC4	*Caulobacteraceae_ge*	QC5	*Xanthomonadaceae_ge*
	Xanthomonadaceae_ge		*Paraherbaspirillum*		*Candidatus_Zinderia*
	Caulobacteraceae_un		*Hephaestia*		*Kinneretia*
	Catenococcus		*Methylophagaceae_ge*		*Methylophagaceae_ge*
	Vibrionaceae_ge		*Marine_Methylotrophic_Group_3*		*Marine_Methylotrophic_Group_3*
SPAL21	*Vibrio*	SPAL33	*Candidatus_Zinderia*	SPAL200	*Candidatus_Zinderia*
	Candidatus_Zinderia		*Catenococcus*		*Spongiibacteraceae_BD1-7_clade*
	Pseudoalteromonadaceae_un		*Vibrionaceae_ge*		*Catenococcus*
	Candidatus_Zinderia		*Candidatus_Zinderia*		*Alteromonadaceae_XY-R5*
	Eionea		*Helicobacteraceae_un*		*Neptuniibacter*
TC3	*Thalassotalea*	TC7	*Alteromonadaceae_XY-R5*	TC10	*Glaciecola*
	Colwelliaceae_ge		*Motilimonas*		*Litoribrevibacter*
	Litorisediminivivens		*Campylobacterales_P6-b51_ge*		*Nitrincolaceae_ge*

685